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硕 士 学 位 论 文

大黄鱼 MIF 蛋白多克隆抗体的
制备及应用研究

The preparation and application of
anti-MIF polyclonal antibody

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缩略语中英文对照表

缩写	英文	中文
aa	Amino acid	氨基酸
Ab	Antibody	抗体
A2	Phospholipase A2	磷脂酶
Ag	Antigen	抗原
AP-1	activator protein-1	核转录因子激活蛋白
bp	Base pair	碱基对
Bis	Bisacrylamide	N'-甲叉丙烯酰胺
BSA	Bovine serum albumen	牛血清白蛋白
cAMP	3'-5'-cyclic adenosine monophosphate	环磷酸腺苷
CD	cluster of differentiation	白细胞分化抗原单位
CD3	cluster of differentiation-3	T 细胞识别抗原的主要识别单位
CD74	cluster of differentiation-74	B 细胞识别抗原的主要识别单位
COX-2	cyclo-oxyge-nase-2	环氧酶-2
CV	ceoefficient of variation	变异系数
CINC-3	cytokine-induced neutrophil chemoattractant-3	中性粒细胞趋化因子-3
CK-1	creatine kinase	肌酸激酶
cPLA2	cytosolic phospholipase A2	胞浆型磷脂酶 A2
d	day	天
DAB	3, 3'-diaminobenzidin	3, 3' -二氨基联苯胺
DIBA	Dot Immunobinding Assay	斑点免疫法
DMSO	dimethyl sulfoxide	二甲基亚砷
EDTA	Ethylene diamine teraacetic acid	乙二胺四乙酸
ELISA	Enzyme linked immunosorbent assay	酶联免疫吸附实验
ER	endoplasmic reticulum	内质网
ERK	extracellularregulatedproteinkinases	蛋白激酶
G ⁺	Gram positive bacteria	革兰氏阳性菌
G ⁻	gram-negative bacterium	革兰氏阴性菌
GST	Glutathione S-transferase	谷胱甘肽硫转移酶
GC	glycocorticoid	糖皮质激素
h	Hour	小时
His	histidine	组氨酸
HRP	Horseradish peroxidaseP	辣根过氧化物酶
IBs	Inclusion bodies	包涵体
IFN	Interferon	干扰素
I _k B α	Inhibitors of nuclear factor kappa b	核转录因子- κ B 抑制蛋白
IPTG	Isopropyl- β -D-Thiogalactopyranoside	异丙基硫代- β -D-半乳糖苷
IgG	Immunoglobulin	免疫球蛋白
IL	interleukin	白细胞介素
Jab1	1c-Jun activation domain-binding protein1	Jun 激活区域-连接蛋白
JNK	c-Jun N-terminal kinase-Jun 氨基末	c 端激酶

缩略语中英文对照表

缩写	英文	中文
Kana	Kanamycin	卡那霉素
kb	Kilo base pair	千碱基对
KDa	Kilodalton	千道尔顿
IFN	Interferon	干扰素
LB	Lurin-Bertani medium	LB 培养基
LPS	lipopolysaccharide	脂多糖
mg	milligram	毫克
min	minute	分钟
MIF	macrophage migration inhibitory factor	巨噬细胞移动抑制因子
MIP-2	Macrophage inflammatory protein 2	巨噬细胞炎性蛋白 2
MAPK	Mitogen-activated protein kinase	丝裂原活化蛋白激酶
MEK	Methyl Ethyl Ketone	甲基乙基酮
MMPs	matrix Metalloproteinases	基质金属蛋白酶
mL	milliliter	毫升
mRNA	Messenger ribonucleic acid	信使 RNA
NC	nitrocellulose filter	硝酸纤维素
NCBI	National center for biotechnology information	美国国家生物信息中心
Ni-NTA	nickel-nitrilotriacetic acid	镍-次氨基三乙酸
OD	Optical density	光密度
PAGE	Polyacrylamide gel electrophoresis	聚丙烯酰胺凝胶电泳
PBS	Phosphate buffer saline	磷酸盐缓冲液
PBST	phosphate buffered solution with 0.05% Tween-20	磷酸盐-吐温 20 缓冲液
PcAb	Polyclonal antibody	多克隆抗体
PGRP	Peptidoglycan recognition protein	肽聚糖识别蛋白
PMSF	Phenylmethyl sulfonyl fluoride	苯甲基磺酰氟
PKA	protein kinase A	蛋白激酶 A
PVDF	Polyvinylidene Fluoride	聚偏二氟乙烯
TNF	Tumor necrosis factor	肿瘤坏死因子
TSST-1	Human toxic shock syndrome toxin	人毒性休克综合征毒素 1
PVDF	Polyvinylidene difluoride	聚偏二氟乙烯
RIA	Radioimmunoassay	放射免疫分析
rpm	Round per minute	每分钟转数
SAS	Saturated ammonium sulfate	饱和硫酸铵
SARS	Severe Acute Respiratory Syndrome	严重急性呼吸综合征
SD	Standard deviation	标准偏差
SDS	Sodium dodecyl sulfate	十二烷基硫酸钠
s	second	秒
SNP	single nucleotide polymorphism	单核苷酸多态性
TBS	Tris balanced saline	Tris 平衡盐溶液
TBST	Tri-buffered saline with Tween-20	Tween-20 的 Tris 盐缓冲液

缩略语中英文对照表

缩写	英文	中文
TEMED	N, N, N', N'-tetra methyl ethylene diamin	N', N, N', N-四甲基乙二胺
TLR	Toll like receptor	Toll 类受体
TNF	Tumor necrosis factor	肿瘤坏死因子
TMB	3' , 3' , 5' , 5' , -tetramethylbenzidine	四甲基联苯胺
TPOR	Thiol-protein Oxidoreductase	硫醇蛋白氧化还原酶
Tris	Tris hydroxymethyl aminomethane	三羟甲基氨基甲烷
μL	microlitre	微升
μg	microgram	微克
UTR	Untranslated region	非翻译区
WB	Western Blot	免疫印迹试验

摘 要

大黄鱼 *Pseudosciaena crocea* (Richardson) 是我国特有的经济鱼种, 随着养殖产量的提高和养殖环境的破坏, 细菌性和病毒性疾病所带来的危害也越来越严重。大黄鱼病害的频频发生势必导致药物的大量使用, 由其所带来的耐药性、药物残留及在环境中扩散已影响到食品和环境公共卫生安全。因此, 研究鱼类免疫机制, 了解鱼体免疫相关的细胞因子功能, 从鱼类自身的抗病能力入手提高鱼类自身的免疫力是未来鱼病防治的重要研究内容。

巨噬细胞移动抑制因子 (macrophage migration inhibitory factor, MIF) 是一种集细胞因子、神经内分泌激素和酶特性于一身的多能效蛋白分子, 参与机体天然免疫和获得性免疫系统的调节, 在机体炎症反应中起着重要作用。目前鱼类 MIF 的功能和炎症致病机理的研究却报道甚少。本文利用 pET-MIF (实验室保存) 表达载体在大肠杆菌中表达, 获得了大黄鱼 MIF 融合蛋白, 开展了蛋白纯化研究, 并制备了 2 种高效价的多克隆抗体, 采用 Western Blot 和免疫组织化学分析了大黄鱼 MIF 的组织分布, 并以纯化的兔抗 MIF 抗体为包被抗体、辣根过氧化物酶 (HRP) 标记的鼠抗 MIF 抗体为酶标抗体初步组装双抗 MIF 夹心检测试剂盒, 获得如下结果:

1. 在大肠杆菌中高效表达 MIF 融合蛋白, 并对原核表达条件进行了优化。30℃, 0.8 mM IPTG 诱导 4~6 h, 即可获得较好的表达效果; MIF 融合蛋白主要以可溶的形式存在于超声波破碎菌体后得到的上清中。

2. 利用 Ni^{2+} -NTA 金属螯合层析柱纯化出的 MIF 融合蛋白, 在非变性条件下经亲和层析和浓缩脱盐纯化后, 获得了单一条带的纯化蛋白, 目的蛋白纯度高达 90% 以上。随后分别免疫兔和鼠, 制备出 2 种抗 MIF 多克隆抗体, 并通过间接 ELISA 及 Western Blot 方法对抗体的效价和特异性进行了分析, 结果显示, 兔抗 MIF 多克隆抗体的效价可达 8.1×10^5 , 鼠抗 MIF 多克隆抗体效价约为 5×10^5 且其与 MIF 抗原具有高度的结合特异性。

3. 应用 MIF 多克隆抗体对大黄鱼各组织中 MIF 的表达情况进行了初步研究。通过 Western Blot 检测出大黄鱼肝脏、肾脏、脾脏、性腺、肠道和脑等组

织中均有 MIF 蛋白的表达,免疫组化的分析结果也表明了 MIF 广泛分布于各组织的免疫相关细胞中,不仅在脾脏、肝脏和肾脏的免疫细胞中大量表达,还在与外界环境相接触的组织,如肠道上皮组织细胞中大量存在。这可能与 MIF 有促进对含内毒素细菌的侦察作用,利于宿主抗微生物防卫系统第一线的细胞更迅速地对入侵的微生物起反应。此外,在脑细胞中也有 MIF 的分泌,暗示着鱼类 MIF 的分泌类似于哺乳动物中 MIF 的分泌过程,即由脑组织直接以“激素样”形式分泌。

4.初步探讨了 MIF 双抗检测试剂盒的制备,第一抗体以 1:2000 包被时,酶标抗体的最佳使用浓度是 1:800。研究结果为今后更好了解 MIF 的生物学功能奠定了重要基础。

5.经蛋白质抗原决定簇预测发现,大黄鱼 MIF 和眼斑拟石首鱼 MIF 具有相同的抗原表位,这为在其它硬骨鱼中广泛应用所获得的抗大黄鱼 MIF 抗体,研究鱼类 MIF 的免疫学功能及抗 MIF 抗体在炎症疾病上的拮抗作用提供了物质基础。

本次实验首次开展了鱼类 MIF 蛋白表达与组织分布的研究,为进一步深入了解鱼类 MIF 在炎症反应中的生物学功能、更清楚了解大黄鱼的抗病分子机制奠定了基础,也为解决大黄鱼养殖病害的免疫防治提供了科学依据。

关键词: 巨噬细胞移动抑制因子 (MIF); 抗体制备; 免疫组织化学; 双抗夹心检测试剂盒

Abstract

Pseudosciaena crocea (Richardson) is an economically important fish. However, with the development of intensive culture, many pathogens have emerged, which caused substantial economic damage. Antibiotics were used to prevent and cure diseases. However, the abuse of antibiotics has made severe effect on food security and public health. Improving its own antipathogen ability and immunologic function is becoming a key research aspect to against fish disease in future study. On the base of understanding the immune system of large yellow croaker such as the mechanism of inflammatory would be an important research direction in fish disease control.

Mammalian macrophage migration inhibitory factor (MIF) is a kind of hormone originated from pituitary with multiple functions in systemic immune and inflammatory. However, Information on the function of MIF in fish immune response is still limited. In present study, the recombinant plasmid pET-MIF was used to express the fusion MIF proteins in *E. coli* BL21 cells (DE3) with IPTG induction, and then used to prepare polyclonal antibody. The polyclonal antibody of rabbit-anti-MIF was used as a marker for immunohistochemical detection of the localization of MIF expression in the main immune organs of large yellow croaker. The results are showed as follows:

1. There was high level expression of 6His-MIF in *E. coli* BL21 cells (DE3) with 0.8 mM IPTG induction under 30°C for 4–6 h. MIF fusion protein was detected in the supernatant of induced cells.
2. Fusion protein was purified to a purity of above 90 % under native condition after His-tag affinity chromatography and desalting, which was used to prepare two kinds of polyclonal antibody. Analysis the binding specificity between MIF and its polyclonal antibody by ELISA and Western Blot. The results showed that the potency of rabbit IgG anti - MIF was 8.1×10^{-5} and the potency of mouse IgG anti - MIF was 5.0×10^{-5} , both of them had high specificity.

3. MIF protein was examined in tissues by Immunohistochemistry and Western Blot analysis, including liver, gonad, spleen, intestine, head kidney and brain. The results demonstrated that MIF was constitutively expressed in all selected tissues in spite that the expression level was somewhat different. MIF was extensively distributed in the tissues that was exposed to external environment such as intestinal epithelial, and it accorded to the function of MIF that it could improve the intestinal mucosal barrier to bacterial endotoxin reinfection. Moreover, fish brain can secrete MIF directly, which has the similar function in that of mammalian.

4. Using rabbit IgG anti-MIF for coated antibody and HRP-mouse IgG anti-MIF for detected antibody to assemble the Double-antibody Sandwich ELISA kits, the optimal dilution of coated antibody and detected antibody was detected to be 1: 2000 and 1: 800, respectively. The method was preliminarily established to detect MIF in fish rapidly and specifically.

5. The comparability of antigenic determinant between large yellow croaker and the red drum suggested that anti-MIF antibody could be used to analysis the distribution of MIF in the red drum. High conservatism of MIF protein, is also an important condition to study MIF immunologic function in other teleost with anti-Large Yellow Croaker MIF antibody.

This is the first study on the distribution of MIF protein in fish, which established a basement for further understanding of biologically essential activity of this molecular and will pave the way for studying its mechanism in the inflammatory disease in large yellow croaker. It provides reference for the establishment of healthy culture technology in large yellow croaker culture.

Key words: macrophage migration inhibitory factor (MIF) ; Preparation Of PolyAntibody ; Immunohistochemistry Assay; Double Antibody Sandwich ELISA kits

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